

REPELLENCY OF CINNAMIC ACID ESTERS TO CAPTIVE RED-WINGED BLACKBIRDS

MICHAEL L. AVERY, USDA/APHIS, Denver Wildlife Research Center, Florida Field Station, 2820 E. University Avenue, Gainesville, FL 32601
DAVID G. DECKER, USDA/APHIS, Denver Wildlife Research Center, Florida Field Station, 2820 E. University Avenue, Gainesville, FL 32601

Abstract: Each year, blackbirds cause millions of dollars of damage to newly planted rice in the southern United States. Currently, there is no product registered as a bird repellent rice seed treatment, so we conducted 2-cup feeding trials with individually caged male red-winged blackbirds (*Agelaius phoeniceus*) to test the repellency of methyl cinnamate and ethyl cinnamate, 2 naturally occurring esters of cinnamic acid. While ethyl cinnamate was moderately deterrent, consumption of treated rice was virtually eliminated by a 1.0% (g/g) application of methyl cinnamate. Additional effort should be given to understanding the chemical and physiological bases of repellency of feeding deterrents such as methyl cinnamate as well as to the development of these materials as bird management tools.

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Damage to newly planted rice by red-winged blackbirds and other species is estimated to exceed \$8 million annually in Texas (Decker et al. 1990) and Louisiana (Wilson et al. 1989) combined. Recent studies to develop lethal (Glahn and Wilson 1992) and nonlethal (Decker et al. 1990, Avery and Decker 1991) control methods have yielded promising preliminary results, but currently no product is registered to control bird depredations of rice.

A potential source of bird deterrent seed treatments is the array of naturally occurring anti-herbivory compounds (e.g., Greig-Smith 1988, Schultz 1988, Jakubas et al. 1989), especially those known to deter seed predators. Cinnamic acid and some of its derivatives inhibit feeding by insects (e.g., Jones and Firn 1979) and birds (Crocker and Perry 1990). Esters of cinnamic acid compose a substantial portion of the volatile chemicals in doveweed (*Eremocarpus setigerus*), a plant widely distributed in western North America that is shunned by deer (Schultz et al. 1980) and whose seeds are avoided by mourning doves (*Zenaida macroura*) and other birds (Cook et al. 1971).

We hypothesized that one or more of the compounds identified from this plant might be responsible for the unpalatability of its seeds to birds. To examine this idea, we treated rice seed with compounds found in doveweed and offered the treated seed to captive red-winged blackbirds, the principal rice-depredating species. We selected methyl and ethyl cinnamate for initial behavioral evaluation because they are structurally the simplest cinnamic acid esters present in doveweed (Schultz et al. 1980).

We appreciate the review comments of J. R. Mason. L. A. Whitehead assisted in preparation of the manuscript. We followed an approved animal welfare protocol during the course of this study.

METHODS General Procedures

We conducted the study at the Florida Field Station of the Denver Wildlife Research Center in Gainesville. Experimental subjects were male red-winged blackbirds, trapped locally and held in captivity 1-3 months before testing. During this time, they had free access to F-R-M® Game Bird Starter (GBS). We conducted the feeding trials in a roofed outdoor aviary, where test cages (45 × 45 × 90 cm) were visually isolated and equipped with automatic waterers. Food was presented in clear plastic feed cups (8.2-cm diameter, 3.8 cm high) with a circular opening (3.1-cm diameter) in the top.

Feeding Trials

Four days before the start of the test, redwings were taken from their holding cages, their mass determined, and they were randomly assigned to test cages. We formed treatment groups (*n* = 5 birds/group) by assigning the bird with the greatest mass to the first group, the one with the next greatest mass to the second group, and so on. After 1 bird was assigned to each treatment group, the order of assignment was reversed. With this procedure, we attempted to equalize consumption among groups. During the 4-day acclimation period, we gave birds 2 food cups

Table 1. Analysis of variance on daily rice consumption (g/cup/bird) by male red-winged blackbirds during 2-cup feeding trials with methyl cinnamate on rough rice, Gainesville, Florida, 1990–91.

Source	df	SS	MS	F	P
Concentration	7	181.6	25.9	0.04	0.897
Error	32	2,088.1	65.3		
Period	1	31.3	31.3	3.1	0.087
Period × concentration	7	58.6	8.4	0.8	0.569
Error	32	321.9	10.1		
Cup	1	288.8	288.8	50.8	<0.001
Cup × concentration	7	438.7	62.7	11.0	<0.001
Error	32	181.8	5.7		
Period × cup	1	175.7	175.7	34.7	<0.001
Period × cup × concentration	7	665.4	95.1	18.8	<0.001
Error	672	3,406.6	5.1		
Total	799	7,838.7			

each containing a mixture of rough (unhulled) rice and GBS.

Following acclimation, there was a 5-day pretreatment period, a 2-day break, and a 5-day treatment period. We assigned 1 cup (cup A) in each cage for treatment at the start of the pretreatment period. The other one, cup B, always held untreated rice. The positions of the cups were switched daily throughout the test. During pretreatment, each food cup contained 30 g of untreated rough rice. In the treatment phase, cup A contained cinnamate-treated rice. We offered methyl cinnamate at 8 concentrations: 0%, 0.005%, 0.01%, 0.05%, 0.1%, 0.4%, 0.7%, and 1.0% a.i. (g/g). We tested ethyl cinnamate at 0%, 0.05%, 0.1%, 0.7%, and 1.0% a.i. (g/g).

Throughout the study, we removed maintenance food at 0800 and, 1 hour later, put in the test food cups. After 6 hours, we replaced cups A and B with 2 cups of the birds' maintenance food (GBS). We determined the mass of the contents of cups A and B on an electric balance and determined consumption by subtraction. After the fifth treatment day, we redetermined mass and released the test birds.

We prepared treated seeds in 1-kg batches by dissolving the appropriate amount of methyl or ethyl cinnamate (99% purity, Aldrich Chemical Co., Milwaukee, Wis.) in 30 mL of acetone and slowly mixing this with the seeds for 10 minutes in a rotating tumbler. Seeds were air-dried for 6 hours and stored in polypropylene bottles in an air-conditioned laboratory.

Analyses

We evaluated results in 2 ways. First, we examined the effects of test period, test cup, and

cinnamate concentration on rice consumption for each ester separately in 3-way repeated measures analyses of variance (ANOVA). Differences ($P = 0.05$) among means were isolated post hoc with the Tukey HSD test (Steel and Torrie 1980). Next, we calculated preference ratios by dividing each bird's total daily consumption (cup A plus cup B) into its consumption from cup A. A value of 0.5 denoted indifference to the treatment, with lower values indicating rejection of the treated food. We plotted ratios for both esters against cinnamate treatment level to examine concentration response patterns. Changes in body mass among the treatment groups were examined in a 1-way ANOVA.

For a total of 6 hours on the first 2 treatment days, we videotaped 1 bird in the 1.0% methyl cinnamate-treatment group. We later viewed the videotape to assess the bird's behavioral response to the treated rice seed.

RESULTS

Methyl Cinnamate Trial

Mean food consumption was not affected by methyl cinnamate concentration ($P = 0.90$) or by test period ($P = 0.09$) (Table 1). Mean food consumption from the untreated cup was greater (6.4 g/bird; $P < 0.001$) than that from the treated cup (5.2 g/bird).

Post-hoc examination of the period × cup interaction ($P < 0.001$) showed that the application of methyl cinnamate to rice in cup A reduced consumption from that cup. Birds in the 1.0% methyl cinnamate group were largely responsible for this effect as evidenced by post-hoc examination of the interaction ($P < 0.001$)

Table 2. Daily rice consumption (g/bird) by red-winged blackbirds ($n = 5$ birds/treatment) in a 2-cup feeding trial with methyl cinnamate, Gainesville, Florida, 1990–91.

Treatment (%, g/g)	Pretreatment ^a				Treatment ^a			
	Cup A		Cup B		Cup A		Cup B	
	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE
0	4.9	0.4	5.4	0.4	4.7	0.3	5.5	0.3
0.005	6.4	0.6	6.5	0.6	6.5	0.4	6.6	0.4
0.01	5.9	0.5	7.1	0.6	5.2	0.4	5.4	0.4
0.05	5.9	0.7	6.8	0.8	5.4	0.5	5.7	0.7
0.1	6.1	0.6	6.5	0.7	4.7	0.6	6.0	0.5
0.4	6.3	0.7	5.9	0.8	5.5	0.5	6.8	0.8
0.7	4.8	0.7	4.7	0.5	3.8	0.4	6.5	0.5
1.0	6.5	0.6	6.1	0.4	0.2	0.1	10.7	0.7

^a During pretreatment, both cups held untreated rice; during treatment, cup A held rice treated with methyl cinnamate.

between cup and concentration. The 3-way interaction ($P < 0.001$) reflected reduced consumption of treated rice by the 0.7% and 1.0% groups (Table 2).

Ethyl Cinnamate Trial

Mean consumption from the treated cup (4.5 g/bird) was less ($P = 0.002$) than that from the untreated cup (5.6 g/bird) (Table 3). Also, mean consumption during the pretreatment phase (5.4 g/bird/cup) was greater ($P = 0.005$) than during the treatment period (4.7 g/bird/cup). Overall, consumption did not differ ($P > 0.7$) among treatment groups (Table 3).

Post-hoc examination of the period \times cup interaction ($P < 0.03$) indicated that consumption from the treated cup decreased in the treatment period. There was no interaction ($P > 0.11$) between cup and concentration. Evaluation of the 3-way interaction ($P < 0.01$) indicated that there was a differential response among the treatment groups during the treat-

ment period. In particular, the 0.7% and 1.0% groups ate less from the treated cup than from the untreated cup ($P < 0.05$) during the treatment period (Table 4).

Dose-Response Relationship

In terms of preference ratios, test birds responded similarly and with indifference to the 2 compounds at lower concentrations (Fig. 1). At 1.0%, however, methyl cinnamate (preference ratio = 0.02) proved markedly more aversive than did ethyl cinnamate (preference ratio = 0.35). Birds exposed to 1.0% methyl cinnamate-treated food rejected it decisively on the first treatment day (Fig. 2), and there was no evidence of habituation.

On the first treatment day, a malfunction of the video camera prevented our viewing the initial reactions of 1 test bird to the 1.0% methyl cinnamate treatment. We did obtain observations 3.5–5.5 hours into the trial. The bird appeared healthy and active throughout this pe-

Table 3. Analysis of variance on daily rice consumption (g/cup/bird) by male red-winged blackbirds during 2-cup feeding trials with ethyl cinnamate on rough rice, Gainesville, Florida, 1990–91.

Source	df	SS	MS	F	P
Concentration	4	183.3	45.8	0.5	0.706
Error	20	1,685.5	84.3		
Period	1	75.2	75.2	10.1	0.005
Period \times concentration	4	27.5	6.9	0.9	0.468
Error	20	148.5	7.4		
Cup	1	143.5	143.5	12.2	0.002
Cup \times concentration	4	101.8	25.5	2.2	0.111
Error	20	236.0	11.8		
Period \times cup	1	44.1	44.1	4.9	0.028
Period \times cup \times concentration	4	128.3	32.1	3.5	0.007
Error	420	3,802.9	9.1		
Total	499	6,576.6			

Table 4. Daily rice consumption (g/bird) by red-winged blackbirds ($n = 5$ birds/treatment) in a 2-cup feeding trial with ethyl cinnamate, Gainesville, Florida, 1990–91.

Treatment (%, g/g)	Pretreatment ^a				Treatment ^a			
	Cup A		Cup B		Cup A		Cup B	
	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE
0	3.9	0.6	4.4	0.6	3.9	0.6	4.2	0.6
0.05	4.7	0.6	5.8	0.6	4.3	0.6	4.2	0.7
0.1	5.7	0.8	6.0	0.7	4.5	0.6	5.0	0.7
0.7	5.9	0.6	5.9	0.8	3.0	0.6	6.1	0.6
1.0	5.8	0.8	6.4	0.8	3.4	0.5	8.1	1.2

^a During pretreatment, both cups held untreated rice; during treatment, cup A held rice treated with ethyl cinnamate.

riod. It fed from the untreated cup 6 times and did not visit the treated cup. During 4 hours of taped observations on the second treatment day, this bird took an occasional seed from the treated cup but displayed no obvious sign of discomfort.

Body Mass of Test Birds

No changes in body mass occurred among test birds in either the methyl cinnamate ($P = 0.241$; $F = 1.40$; 7, 32 df) or the ethyl cinnamate ($P = 0.914$; $F = 0.24$; 4, 20 df) trial. For individual birds in the methyl cinnamate trial, changes in mass ranged from a loss of 4.5 g to a gain of 7.5 g. Among ethyl cinnamate birds, extremes were a loss of 3.5 g and a gain of 2.0 g.

DISCUSSION

Although differences in experimental designs require that comparisons among studies be made cautiously, at 1.0% (g/g), methyl cinnamate

compares favorably with other avian feeding deterrents evaluated under similar conditions. For example, we obtained mean preference ratios of 0.05–0.09 in tests of redwings feeding on rough rice treated with various fungicides (Avery and Decker 1991). Group-tested redwings exhibited preferences of 0.05 and 0.22 when offered food treated with 1.0% (g/g) methyl anthranilate and dimethyl anthranilate, respectively (Avery et al. 1988). In 2-cup tests involving food adulterated with aminoacetophenones, individually caged European starlings (*Sturnus vulgaris*) exhibited preference ratios of 0.10–0.25 to the 1.0% (g/g) treatments (ratios estimated from Figs. 2–5 in Mason et al. 1991b). Jakubas et al. (1992) evaluated the repellency of several benzoic acid and cinnamic acid derivatives in 2-cup feeding trials with starlings and found that only 3,4-dimethoxycinnamyl alcohol produced a preference ratio below 0.1 at a concentration near 1.0% (g/g).

Although the basis for the redwings' rejection

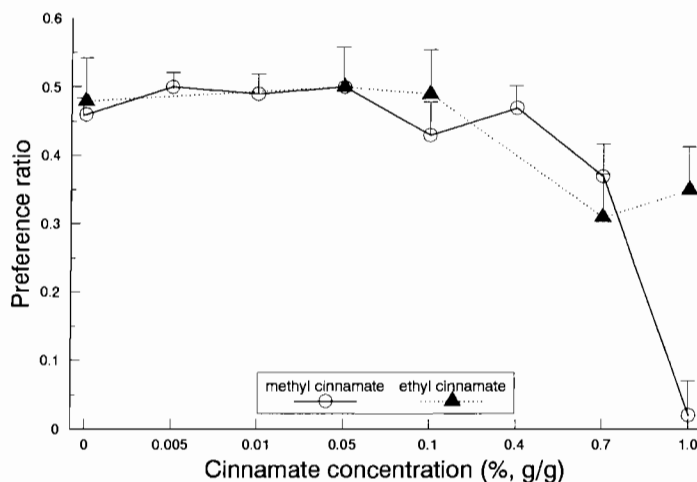


Fig. 1. Mean preference ratios (treated food consumption divided by consumption of treated plus untreated food) of red-winged blackbird groups in 2-cup tests with rough rice treated with cinnamate esters at various concentrations. Capped bars indicate 1 SE. A preference ratio of 0.5 indicates indifference; lower scores indicate avoidance.

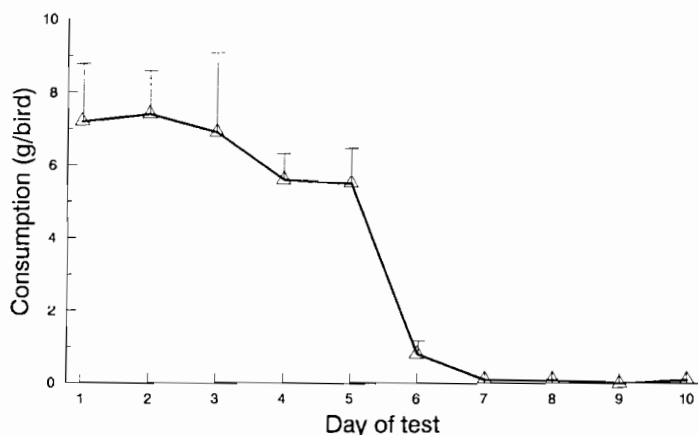


Fig. 2. Mean daily consumption of rough rice from cup A by red-winged blackbirds. During days 1–5, cup A held untreated rice; on days 6–10 cup A held rice treated with 1.0% (g/g) methyl cinnamate. Capped bars indicate 1 SE.

of methyl cinnamate-treated rice is unclear, we speculate that the birds' response was mediated by taste, olfaction, or trigeminal chemoreception. The repellency of methyl anthranilate and related compounds is trigeminally mediated (Mason et al. 1989), and has a temporal response pattern similar to that of methyl cinnamate (Mason et al. 1991a). Avoidance of the treated rice from the first treatment day onward suggests that the birds' response required, at most, a very short learning period. Our limited videotape observations support this view; within 3.5 hours of its initial exposure, the focal bird was avoiding the treated food cup.

Although it is tempting to attribute methyl cinnamate repellency to trigeminal or olfactory irritation, we cannot rule out a component of repellency due to postingestional effects. Crocker and Perry (1990) postulated that inhibition of digesting enzymes was the basis for feeding deterrence produced by the cinnamic acid derivatives they tested. Biochemical assays failed to confirm this supposition, however. The rapid rejection of methyl cinnamate-treated rice suggests behavior based on immediate sensory feedback, but if ingested, it is possible that methyl cinnamate could produce negative effects in the gut. Jakubas and Gullion (1990) made similar observations about coniferyl benzoate.

RESEARCH AND MANAGEMENT IMPLICATIONS

Additional study is needed to explain the mechanism for the feeding deterrence elicited

by methyl cinnamate. Such work may include more detailed monitoring of temporal food consumption patterns and detailed behavioral observations to detect sensory responses and post-ingestional reactions to treated food. Because cinnamic acid can be converted microbially to acetophenone (Hilton and Cain 1990), the possibility arises that the bird repellent activities of cinnamic acid derivatives (Crocker and Perry 1990), aminoacetophenones (Clark and Shah 1991, Mason et al. 1991b), and related compounds (Jakubas et al. 1992) have a common chemical basis.

Despite its effectiveness as a blackbird feeding deterrent, and its status as an approved food additive (Hall and Oser 1965, Winter 1972), the future of methyl cinnamate as a registered bird repellent is unclear. Registration of pesticides, including bird repellent compounds, is costly (Fagerstone et al. 1990), and the small market for such products limits profitability (Tobin and Dolbeer 1987). Because of health concerns, there are greater data requirements and considerably higher registration costs for food crop uses than for nonfood uses (Fagerstone et al. 1990). Thus, the prospects for commercial development of newly identified bird repellents are probably better in nonfood applications than they are in food crops. In the continued development and testing of new candidate repellents such as methyl cinnamate, there may be merit in specifically targeting nonfood use patterns first. Conversely, in food crops, it might be more pragmatic to identify bird repellent properties

of currently registered chemicals and expand their uses (Avery and Decker 1991). While this approach will not identify optimal bird repellent compounds, it will enhance their commercial availability, thereby producing useful tools than can be rapidly incorporated into integrated bird management programs.

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